Lipid Amphiphiles

Dioxazocinium Ortho Esters: A Class of Highly pH-Vulnerable Amphiphiles**

*Kolbot By and Michael H. Nantz**

Micelles and vesicles derived from pH-sensitive amphiphiles continue to attract attention for a variety of applications.[1,2] Of particular recent interest is the use of cationic, pH-sensitive liposomes as delivery vehicles for DNA therapeutics.[3] Our interest in lipids that disassemble on exposure to mild acid conditions, such as the pH 5–6 environment within endosomes,[4] led us to engineer lipids that feature the highly pH-vulnerable ortho ester moiety.[5] We disclose herein the synthesis of an even more sensitive class of pH-vulnerable lipids from a novel dioxazocinium ketene acetal reagent. The results of formulation and hydrolysis studies with representative lipids **1**–**4** (Scheme 1) and a single-chain analogue are presented to illustrate the versatility of the synthesis and pH sensitivity.

[*] K. By, Prof. M. H. Nantz
Department of Chemistry
University of California, Davis, CA 95616 (USA)
Fax: (+1) 530-752-8995
E-mail: mhnantz@ucdavis.edu

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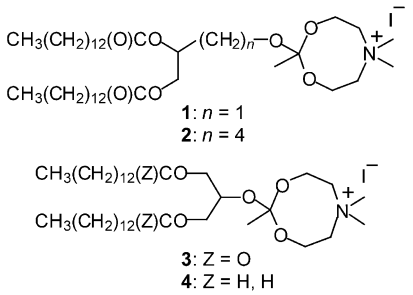
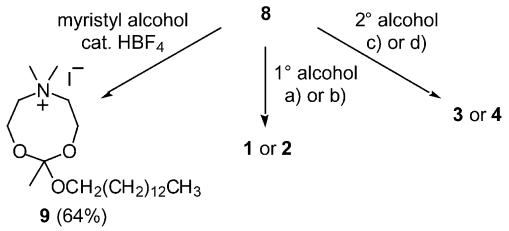
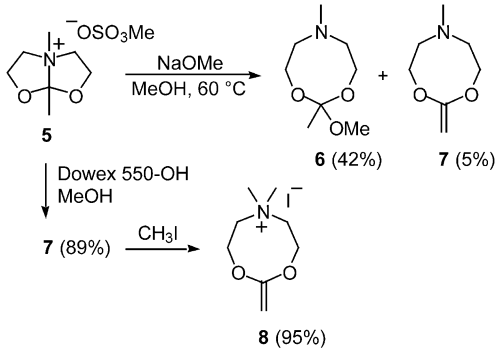


Scheme 1. Dioxazocinium ortho ester lipids.



Scheme 3. Ortho ester synthesis: a) 1,2-ditetradecanoyl-*rac*-glycerol, DMF, 69% (**1**); b) 1,2-dimyristoyloxy-6-hexanol, CH_2Cl_2:DMF (3:1), 86% (**2**); c) 1,3-ditetradecanoyl-*rac*-glycerol, DMF, 61% (**3**); d) 1,3-dimyristyloxy-2-propanol, CH_2Cl_2:DMF (3:1), 43% (**4**). DMF = *N*,*N*-dimethylformamide.

Lipid synthesis: Exposure of ammonium salt **5**[6] (Scheme 2) to NaOMe gives rise to methyl ortho ester **6** and to ketene acetal **7** as the consequence of a competing Hofmann-type elimination.[7] We reasoned that the ketene acetal moiety in **7** could serve as a progenitor of amphiphilic ortho esters. Hence, we aimed to optimize the yield of **7** from **5**. Our initial efforts resulted in a protocol in which sodium



Scheme 2. Synthesis of the ketene acetal reagent **8**.

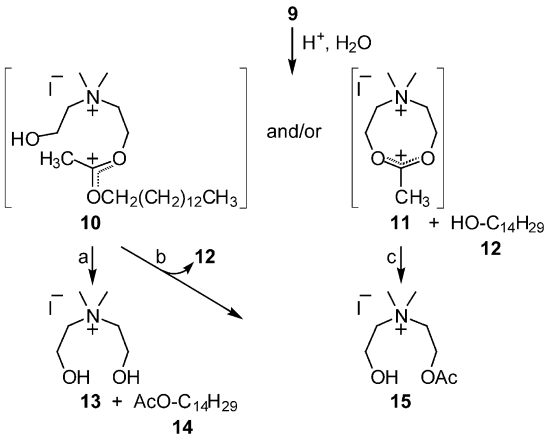
triphenylmethoxide and **5** were heated under vacuum in tetrahydrothiophene 1,1-dioxide (sulfolane). Ketene acetal **7** was collected by direct distillation. Final purification afforded a 36% yield of pure **7** on a 20-gram scale. Ultimately, we discovered that simple elution of **5** as a methanol solution through an anion-exchange resin gave ketene acetal **7** in 89% yield. Although hydroxide ion exchange resins are often used to prepare ammonium hydroxide salts, temperatures in excess of 100 °C are typically required to effect subsequent Hofmann eliminations.[8] Thus the formation of **7** under these mild conditions represents a convenient but unusual ketene acetal synthesis.[9]

Ortho ester formation with **7** proved to be sluggish without the assistance of an acid catalyst and low yielding when only catalytic amounts of acid were employed. However, quaternization of **7** cleanly afforded **8** (Scheme 2). In contrast to **7**, ammonium salt **8** can be stored without decomposition[10] and readily converted into ortho esters (Scheme 3). The synthesis is general. For example, under HBF_4 catalysis, reaction of **8** with myristyl alcohol provided ortho ester **9**. Other catalysts were examined (trifluoromethanesulfonic acid, methanesulfonic acid, anhydrous HCl in diethyl ether), but none were as effective as HBF_4. Reaction of **8** with dual-chain substituted primary and secondary alcohols gave the ortho esters **1**–**4** in good to moderate yields depending on the degree of substitution of the alcohol.

Lipid properties: With the dioxazocinium lipids prepared, we turned our attention toward liposome formulation and pH-vulnerability issues. Incorporation of dioloeylphosphatidylethanolamine (DOPE) during the hydration and sonication of the dual-chain analogues **1**–**4** greatly improved the ability of these lipids to assemble in water.[11] In separate D_2O experiments, ^1H NMR analysis showed that sonication and heating of **1**–**4** at 50 °C for 1 h did not hydrolyze the ortho ester. Transmission electron microscopy (TEM) analyses of representative preparations derived from **1** and **3** revealed multilamellar lipid aggregates with sizes of 95–220 nm.[12] Light-scattering-particle size measurements also indicated aggregates with mean diameters of 165 and 159 nm for the DOPE preparations of **1** and **3**, respectively.

To examine the susceptibility of the dioxazocinium ortho ester linkage toward acid-induced cleavage under different conditions, we considered the case of the single-chain derivative **9**. Ortho ester hydrolysis is believed to proceed through a three-stage reaction mechanism that involves, as the first stage, the generation of a dioxocarbenium ion intermediate.[13] Though many variables are involved in the hydrolysis,[14] cyclic ortho esters form dioxocarbenium ions either by endocyclic C–O or exocyclic C–O bond cleavage, with the latter being the preferred mode in small to medium-size rings.[15] In the case of **9**, endocyclic C–O bond cleavage would give rise to dioxocarbenium species **10** (Scheme 4), while heterolysis of an exocyclic C–O bond would give **11** with concomitant formation of myristyl alcohol (**12**). Subsequent interception of these dioxocarbenium species by water results in products from paths a–c. While **10** may serve as a precursor to diol **13** (path a), the monoacetate **15** (path b), and the accompanying myristyl hydrolysates **14** and **12**, respectively, the cyclic species **11** can form only monoacetate **15**. This mechanistic interpretation suggests that the appearance of the myristyl hydrolysis products could be used as a convenient probe for monitoring the progress of the hydrolysis.

Incubation of **9** in HOAc/NaOAc buffer solutions at pH 5.0, 5.5, and 6.0 with monitoring by GC/MS to quantify the appearance of **12** and **14** revealed that myristyl acetate was the predominant by-product, thereby implicating path a as the principal mode of hydrolysis.[12] Only trace amounts of



Scheme 4. Hydrolysis of the ortho esters.

myristyl alcohol (**12**, $<5\%$) were detected in these experiments. The data (Figure 1) suggest an apparent zero-order rate law consistent with the acid-catalyzed dissociative mechanism reported for ortho ester hydrolysis.[13] The rate of hydrolysis of **9** is faster than that of other cationic lipids—**9** was completely hydrolyzed at pH 5.0 within six hours ($t_{1/2} = 120 \pm 1$ min). Extrapolation of the linear plot data for the pH 5.5 and 6.0 conditions gave $t_{1/2}$ values of 250 ± 17 and 407 ± 15 min, respectively.

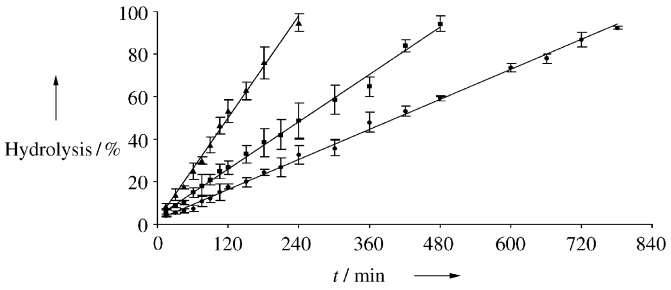


Figure 1. Linear plots for the hydrolysis of **9** in HOAc/NaOAc buffer solutions at pH 5.0 (▲), pH 5.5 (■), and pH 6.0 (●). Hydrolyses were performed at 38 °C in the presence of 1-methoxydodecane as an internal standard. Each data point represents the average of three experiments; the bars for each point represent the standard deviation.

The high sensitivity of **9** toward acid-catalyzed cleavage can be attributed to a combination of structural features. The flexibility imparted by the eight-membered ring of **9** increases the possibility for either *anti*- or *syn*-periplanar dissociation[15] to the dioxocarbenium species **10**. The proximal ammonium group may also facilitate formation of the dioxocarbenium ion by improving the leaving-group ability of the endocyclic oxygen atoms. While this hypothesis is consistent with the interpretation of the rate study, the details of the factors that govern the hydrolysis of dioxazocinium ortho esters have yet to be confirmed.

Substitution of the ortho ester influences the rate of hydrolysis. Exposure of the liposome suspensions derived from lipids **1**–**4** to pH 5.0 for 6 h at 38 °C revealed that the ortho ester moieties in **1**, **3**, and **4** had undergone only partial (approximately 10–50 %) hydrolysis. While the hydrolysis rate certainly is influenced by differences in the aggregation state (that is, micellar with **9**, lamellar with **1**–**4**), substituent effects also contribute. Lipid **3** is the slowest to hydrolyze, a fact which is consistent with the expected electron-withdrawing influences of the β-acyloxy and β-alkoxy groups. Lipid **2**, which closely approximates the alkyl side-chain structure of **9**, is the most sensitive of the dual-chain amphiphiles. In competition hydrolysis experiments of **9** mixed with each dual-chain lipid we found that **9** was clearly more vulnerable except when mixed with **2**, where we observed essentially no difference in the rate of hydrolysis between **2** and **9**. The ortho ester moiety in **2** undergoes complete hydrolysis at pH 5.0 within 6 h. These results show that subtle structural differences can be used to adjust the pH sensitivity of a dioxazocinium ortho ester. This facet may be useful in tailoring amphiphiles of this type to cleave at a targeted, mild pH value.

In summary, we have reported an efficient synthesis of a dioxazocinium ketene acetal that can serve as a reagent for the transformation of hydrophobic primary or secondary alcohols into pH-vulnerable ortho ester amphiphiles. Hydrolysis studies revealed unprecedented pH-sensitivity among the cationic lipids and provided preliminary indications that hydrolysis of dioxazocinium ortho esters occurs through the endocyclic C–O bond cleavage mode.

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[1] a) C. Wang, Q. Gao, J. Huang, *Langmuir* **2003**, *19*, 3757; b) Y. Rui, S. Wang, P. S. Low, D. H. Thompson, *J. Am. Chem. Soc.* **1998**, *120*, 11213; c) D. A. Jaeger, B. Li, T. Clark, Jr., *Langmuir* **1996**, *12*, 4314; d) D. Ono, S. Yamamura, M. Nakamura, T. Takeda, A. Masuyama, Y. Nakatsuji, *J. Am. Oil Chem. Soc.* **1995**, *72*, 853; e) D. A. Jaeger, J. Jamrozik, T. G. Golich, M. W. Clennan, J. Mohebalian, *J. Am. Chem. Soc.* **1989**, *111*, 3001.

[2] For a recent review on liposome delivery, see: T. Lian, R. J. Y. Ho, *J. Pharm. Sci.* **2001**, *90*, 667.

[3] a) J. S. Choi, J. A. MacKay, F. C. Szoka, Jr., *Bioconjugate Chem.* **2003**, *14*, 420; b) J. A. Boomer, D. H. Thompson, S. M. Sullivan, *Pharm. Res.* **2002**, *19*, 1289; c) E. Liang, M. N. Rosenblatt, P. S. Ajmani, J. A. Hughes, *Eur. J. Pharm. Sci.* **2000**, *11*, 199.

[4] J. A. Reddy, P. S. Low, *J. Controlled Release* **2000**, *64*, 27.

[5] J. Zhu, R. J. Munn, M. H. Nantz, *J. Am. Chem. Soc.* **2000**, *122*, 2645.

[6] a) K. Burzin, R. Feinauer, *Angew. Chem.* **1973**, *85*, 1055; *Angew. Chem. Int. Ed. Engl.* **1973**, *12*, 996; b) R. Feinauer, *Angew. Chem.* **1968**, *80*, 703; *Angew. Chem. Int. Ed. Engl.* **1968**, *7*, 731.

[7] R. Feinauer, *Synthesis* **1971**, 16.

[8] For example, see: C. Kaiser, J. Weinstock, *Org. Synth.* **1976**, *55*, 3.

[9] a) S. M. McElvain, L. R. Morris, *J. Am. Chem. Soc.* **1951**, *73*, 206; b) W. J. Bailey, L.-L. Zhou, *Tetrahedron Lett.* **1991**, *32*, 1539; c) L. H. Trogen, *Acta Chem. Scand.* **1993**, *47*, 841.

[10] Analysis of a sample of **8** that had been stored for six months at room temperature revealed no decomposition.

[11] As expected, single-chain analogue **9** did not form lamellate structures.

[12] See Supporting Information for TEM images and representative GC chromatographs.

[13] a) M. Ahmad, R. G. Bergstrom, M. J. Cashen, Y. Chiang, A. J. Kresge, R. A. McClelland, M. F. Powell, *J. Am. Chem. Soc.* **1979**, *101*, 2669; b) C. A. Bunton, R. H. De Wolfe, *J. Org. Chem.* **1965**, *30*, 1371.
[14] a) P. Deslongchamps, Y. L. Dory, L. Shigui, *Tetrahedron* **2000**, *56*, 3533; b) S. Li, A. J. Kirby, P. Deslongchamps, *Tetrahedron Lett.* **1993**, *34*, 7757.
[15] S. Li, Y. L. Dory, P. Deslongchamps, *Tetrahedron* **1996**, *52*, 14841.

